WEST Search History

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DATE: Friday, July 02, 2004

Hide?	Set Name	Query	Hit Count
	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ		
☐	L1	6506559.pn.	2
☐	L2	wo 99/32619	105
☐	L3	wo 01/75164	36
☐	L4	wo 95/34668	13
☐	L5	dicer	1348
☐	L6	dicer activity	21
☐	L7	argonaut or argonaut activity	463
☐	L8	Beach-D$.in. or Bernstein-E$.in. or caudy-A$.in. or Hannon-G$.in.	543
☐	L9	double stranded RNA or dsRNA	6255
☐	L10	guide RNA	82
☐	L11	(attenuat$ near expression same target)	55
☐	L12	((reduc$ or weak$ or less$) near expression same target)	802
☐	L13	L9 same cell	3260
☐	L14	L13 and (L11 or L12)	143
☐	L15	(double stranded RNA or ds RNA) near vector	9
☐	L16	(double stranded RNA or ds RNA) same vector	1223
☐	L17	L16 and L14	34
☐	L18	L13 and hairpin	553
☐	L19	L18 and L14	87
☐	L20	L19 and (complementary near target)	34
☐	L21	hybridiz$ and L20	31
☐	L22	hybridiz$ and L17	28
☐	L23	(L14 and (pathogen gene))	7
☐	L24	L23 and genom$	6
☐	L25	L24 and (whole animal or non-human)	4
☐	L26	L14 and (whole animal or non-human)	69
☐	L27	L14 and genom$	128
☐	L28	L27 and primate cell	8
☐	L29	L14 and (protein kinase RNA activated sequence independent response)	0
☐	L30	L14 and (protein kinase RNA same response)	4

☐	L31	L14 and (protein kinase RNA)	4
☐	L32	(l5 or l6) and l9	128
☐	L33	L32 and ((reduc$ or weak$ or less$ or inhibit$ or attenuat$) same (expression near target))	63
	DB=EPAB; PLUR=YES; OP=ADJ		
☐	L34	WO-9932619-A1.did.	1
	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ		
☐	L35	l33 and vector	59
☐	L36	L35 and protein kinase	25
☐	L37	l14 and protein kinase	47
☐	L38	(l5 or L6) and l7	9
☐	L39	L7 and l9	9

END OF SEARCH HISTORY

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=> d his

     (FILE 'HOME' ENTERED AT 07:58:37 ON 02 JUL 2004)

     FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
     ENTERED AT 07:58:48 ON 02 JUL 2004
L1          4342 S BEACH D?/AU OR BERNSTEIN E?/AU OR CAUDY A?/AU OR HANNON G?/AU
L2         38688 S DOUBLE STRAND## RNA
L3         32979 S  EXPRESS? (5A) TARGET
L4         13895 S L3 AND (ATTENUAT? OR REDUC? OR WEAK? OR LESS? OR INHIBIT?)
L5           247 S L4 AND L2
L6             7 S L5 AND (COMPLEMENT?  AND PORTION)
L7            45 S L5 AND COMPLEMENT?
L8           197 S L5 AND (CELL OR ANIMAL OR PATHOGEN OR GENOME OR PRIMATE)
L9           113 S L1 AND L2
L10            4 S L9 AND L5
L11            4 S L9 AND L4
L12           61 S L8 AND VECTOR
L13           22 S L12 AND HAIRPIN
L14           25 S L8 AND PROTEIN KINASE
L15           52 DUP REM L12 (9 DUPLICATES REMOVED)
L16           18 DUP REM L13 (4 DUPLICATES REMOVED)
L17           17 DUP REM L14 (8 DUPLICATES REMOVED)
L18           37 DUP REM L7 (8 DUPLICATES REMOVED)
L19            7 DUP REM L6 (0 DUPLICATES REMOVED)
L20          144 DUP REM L5 (103 DUPLICATES REMOVED)
L21          108 S L20 AND CELL
L22           45 S L21 AND VECTOR
L23           45 DUP REM L22 (0 DUPLICATES REMOVED)
L24           32 S L20 AND COMPLEMEN?

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(FILE 'HOME' ENTERED AT 10:18:26 ON 02 JUL 2004)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
ENTERED AT 10:18:34 ON 02 JUL 2004

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L1          614 S DICER OR DICER ACTIVITY
L2          172 S ARGONAUT OR ARGONAUT ACTIVITY
L3          290 S (L1 OR L2) AND (DOUBLE STRAND## RNA)
L4           10 S L3 AND (EXPRESSION (5A) TARGET)
L5          159 DUP REM L3 (131 DUPLICATES REMOVED)
L6           24 S L5 AND COMPLEMENT?
L7            5 S L1 AND L2
L8            5 S L2 AND RNA
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                   and June 2004
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 NEWS  6  May 12   Polymer links for the POLYLINK command completed in REGISTRY
 NEWS  7  May 17   FRFULL now available on STN
 NEWS  8  May 27   New UPM (Update Code Maximum) field for more efficient patent
                   SDIs in CAplus
 NEWS  9  May 27   CAplus super roles and document types searchable in REGISTRY
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 NEWS 12  Jun 28   Additional enzyme-catalyzed reactions added to CASREACT
 NEWS 13  Jun 28   ANTE, AQUALINE, BIOENG, CIVILENG, ENVIROENG, MECHENG,
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               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 26 APRIL 2004
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=> file .biotech caplus
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21

FILES 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
ENTERED AT 07:58:48 ON 02 JUL 2004
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.
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7 FILES IN THE FILE LIST

=> s Beach D?/au or bernstein E?/au or caudy A?/au or hannon G?/au
L1        4342 BEACH D?/AU OR BERNSTEIN E?/AU OR CAUDY A?/AU OR HANNON G?/AU

=> s double strand## RNA
L2       38688 DOUBLE STRAND## RNA

=> s  express? (5a) target
L3       32979 EXPRESS? (5A) TARGET

=> s l3 and (attenuat? or reduc? or weak? or less? or inhibit?)
   6 FILES SEARCHED...
L4       13895 L3 AND (ATTENUAT? OR REDUC? OR WEAK? OR LESS? OR INHIBIT?)

=> s l4 and l2
L5         247 L4 AND L2

=> s l5 and (complement?  and portion)
L6           7 L5 AND (COMPLEMENT? AND PORTION)

=> s l5 and complement?
L7          45 L5 AND COMPLEMENT?

=> s l5 and (cell or animal or pathogen or genome or primate)
L8         197 L5 AND (CELL OR ANIMAL OR PATHOGEN OR GENOME OR PRIMATE)

=> s l1 and l2
L9         113 L1 AND L2

=> s l9 and l5
L10          4 L9 AND L5

=> s l9 and l4
L11          4 L9 AND L4

=> s l8 and vector
L12         61 L8 AND VECTOR

=> s l12 and hairpin
L13         22 L12 AND HAIRPIN

=> s l8 and protein kinase
L14         25 L8 AND PROTEIN KINASE

=> d his

     (FILE 'HOME' ENTERED AT 07:58:37 ON 02 JUL 2004)

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     ENTERED AT 07:58:48 ON 02 JUL 2004
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L5          247 S L4 AND L2
L6            7 S L5 AND (COMPLEMENT?  AND PORTION)
L7           45 S L5 AND COMPLEMENT?
L8          197 S L5 AND (CELL OR ANIMAL OR PATHOGEN OR GENOME OR PRIMATE)
L9          113 S L1 AND L2
L10           4 S L9 AND L5
L11           4 S L9 AND L4
L12          61 S L8 AND VECTOR
L13          22 S L12 AND HAIRPIN
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L14 25 S L8 AND PROTEIN KINASE

=> dup rem l12
PROCESSING COMPLETED FOR L12
L15 52 DUP REM L12 (9 DUPLICATES REMOVED)

=> dup rem l13
PROCESSING COMPLETED FOR L13
L16 18 DUP REM L13 (4 DUPLICATES REMOVED)

=> dup rem l14
PROCESSING COMPLETED FOR L14
L17 17 DUP REM L14 (8 DUPLICATES REMOVED)

=> dup rem l7
PROCESSING COMPLETED FOR L7
L18 37 DUP REM L7 (8 DUPLICATES REMOVED)

=> dup rem l6
PROCESSING COMPLETED FOR L6
L19 7 DUP REM L6 (0 DUPLICATES REMOVED)

=> d ibib abs l19 1-7

L19 ANSWER 1 OF 7 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2004-09226 BIOTECHDS
TITLE: Producing an hsiRNA mixture, useful for gene silencing, comprises digesting a preparation of large **double-stranded RNA** in a reaction mixture containing a divalent transition metal cation (e.g. manganese) and RNAseIII; for use as a silencer and in gene targeting and cloning
AUTHOR: TZERTZINIS G; FEEHERY G; TUCKEY C; NOREN C; MCREYNOLDS L
PATENT ASSIGNEE: NEW ENGLAND BIOLABS INC
PATENT INFO: WO 2004015062 19 Feb 2004
APPLICATION INFO: WO 2003-US22540 18 Jul 2003
PRIORITY INFO: US 2003-467541 2 May 2003; US 2002-402769 12 Aug 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-180650 [17]
AN 2004-09226 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Producing an hsiRNA mixture comprising digesting a preparation of large **double-stranded RNA** in a reaction mixture containing a divalent transition metal cation and RNaseIII, and producing the hsiRNA mixture, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) silencing or **reducing expression** of a **target** gene; (2) a set of **double-stranded RNA** fragments, comprising a plurality of overlapping fragments of a size of about 5-30 nucleotides, the fragments in the set collectively representing a substantial **portion** of a sequence of one or more large double-stranded RNAs from which the fragments are derived by in vitro cleavage with a purified enzyme, one strand of each of the large **double-stranded RNA** having a sequence **complementary** to part or all of a target messenger RNA; (3) creating a library of DNA clones from an hsiRNA mixture, each clone expressing one or more **double-stranded RNA** fragments from the hsiRNA mixture; (4) a kit for preparing an hsiRNA mixture, comprising a preparation of RNAseIII, and an RNAse buffer containing manganese ions in the range of about 5-100 mM and, optionally, reagents for synthesizing a large **double-stranded RNA**; (5) obtaining a large **double-stranded RNA** molecule; (6) a rapid discovery method for identifying an

hsiRNA mixture capable of increased gene silencing of a target gene; and (7) identifying a sequence corresponding to an siRNA from a cleavage site in an mRNA

BIOTECHNOLOGY - Preferred Method: In producing an hsiRNA mixture, the hsiRNA mixture is the product of complete digestion of the preparation of large **double-stranded RNA**. A w/w ratio of RNAseIII to large **double-stranded RNA** in the reaction mixture is in a range of about 0.005:1 to 25:1 or about 0.0125:1 to 10:1. The transition metal cation is manganese. The reaction mixture contains manganese ions at a concentration in the range of about 5-10 or 10-20 mM. The transition metal is selected from nickel, cobalt and cadmium. The complete digestion is achieved in **less** than 6 hours, preferably **less** than 2 hours. The method comprises digesting a preparation of large **double-stranded RNA** in a reaction mixture containing RNaseIII in a ratio of enzyme to substrate (w/w) being greater than or equal to about 0.25:1; and producing the hsiRNA mixture. Silencing **expression** of a **target** gene comprises introducing into a host cell, an hsiRNA mixture produced by the above method, where the nucleotide sequence for each siRNA in the mixture has a sequence that is **complementary** to the target gene. Creating a library of DNA clones from an hsiRNA mixture, each clone expressing one or more **double-stranded RNA** fragments from the hsiRNA mixture, comprises denaturing the hsiRNA mixture to form a mixture of unpaired RNA strands; ligating to a 3' end of the unpaired RNA strands, a first single-stranded DNA primer and to a 5' end of the unpaired RNA strand, a second single-stranded DNA primer; reverse transcribing the chimeric DNA-RNA products to form **complementary** DNA fragments; and inserting one or more DNA fragments into a vector to form the library of clones. The reverse transcribing step further comprises performing a polymerase dependent amplification of the DNA fragments. The 5' end of the RNA strand in the ligating step is dephosphorylated. The 3' end of the RNA strand in the ligating step is a 3' hydroxyl end and where the first DNA primer has both a 5' and a 3' phosphate, the first primer being ligated to the 3' end prior to the second primer. The RNA strand ligated to the first primer is phosphorylated and ligated to the second primer, where the second primer is non-phosphorylated on the 3' ends. Alternatively, creating a library of clones, each clone corresponding to one or more **double-stranded RNA** fragments from an hsiRNA mixture, comprises denaturing the hsiRNA mixture to form a mixture of unpaired RNA strands; enzymatically removing the 5' phosphate from each strand in the mixture; ligating to the 3' hydroxyl end of each strand a DNA primer having both a 5' and a 3' phosphate; enzymatically phosphorylating the 5' end of the resulting species; ligating to the 5' phosphorylated end of each strand, a second DNA primer having non-phosphorylated 3' termini; reverse transcribing the chimeric DNA-RNA products of the ligating step to form **complementary** DNA fragments; and inserting one or more DNA fragments into a vector to form the library of sequences. The reverse transcribing step further comprises performing polymerase-dependent amplification of the DNA fragments. The vector is pUC19 or a Litmus vector. Obtaining a large **double-stranded RNA** molecule comprises inserting a DNA fragment or library of DNA fragments encoding a **double-stranded RNA** into a vector having cloning sites flanked by opposing T7 promoters; performing in vitro or in vivo transcription; and obtaining the large **double-stranded RNA** molecule. **Reducing expression** of one or more **target** genes in a eukaryotic cell comprises introducing into the cell, a set of hsiRNA fragments cited above, where the large dsRNA is **complementary** to all or part of a messenger RNA transcript of each of the **target** genes; and **reducing** the **expression** of the one or more **target** genes in the eukaryotic cell compared to expression of the genes in the eukaryotic cell without the hsiRNA. Alternatively, **reducing**

expression of one or more **target** genes in a eukaryotic cell comprises introducing into the cell, one or more DNA clones made by the above method, where the DNA clones express siRNA fragments suitable for **reducing expression** of the **target** eukaryotic cell compared to **expression** of the genes in the eukaryotic cell without the DNA sequences. The eukaryotic cell is present in a mammal such that **reducing expression** of the one or more **target** genes cause a phenotypic change. The phenotypic change provides a treatment for a disease in the mammal. The phenotypic change is an enhancement of a desired characteristic in the mammal. It is also diagnostic for a selected phenotype. The **reduced** expression of a gene is a tool for analyzing a biochemical pathway in which the gene product functions. The biochemical pathway may be further analyzed in combination with a diagnostic reagent. The diagnostic reagent is an antibody. The eukaryotic cell is present in a non-human animal. The eukaryotic cell that is a component of a transgenic animal is created from a fertilized oocyte containing the DNA sequence. The rapid discovery method for identifying an hsiRNA mixture capable of increased gene silencing of a target gene comprises synthesizing a plurality of large dsRNAs, each large dsRNA having a sequence **complementary** to a segment of a target gene; digesting each of the large dsRNA with RNaseIII in the presence of manganese ions to produce a corresponding hsiRNA mixture; introducing each hsiRNA mixture into a eukaryotic cell to determine whether gene silencing occurs; and determining which of the hsiRNA mixtures caused increased gene silencing. The determining step further comprises combining a first hsiRNA mixture with a second hsiRNA mixture for increasing gene silencing. The method further comprises selecting individual siRNA fragments from hsiRNA mixtures and introducing the individual siRNA fragments into a eukaryotic cell to achieve desired gene silencing. Identifying a sequence corresponding to siRNA from a cleavage site in an mRNA comprises obtaining an hsiRNA mixture enzymatically; introducing the hsiRNA into a cell; extracting cleaved mRNA from the cell; determining the sequence of terminal nucleotides at the cleavage site of the siRNA cleaved mRNA; and identifying the siRNA sequence from the cleavage site sequence and neighboring nucleotides from the intact mRNA. The step of determining the sequence further comprises using labeled extension DNA primers. Preferred Set: The substantial **portion** in the set of fragments is greater than about 50 or 65% of the sequence of the large **double-stranded** **RNA**. More than about 30% of the RNA fragments have a fragment size of about 18-25 base pairs. At least one fragment and as many as 100% of fragments in the set are capable of causing cleavage of the target mRNA in a cell. Preferably, at least about 50 or 75% of the fragments are capable of causing cleavage of the mRNA. The set of fragments is capable of RNA silencing in vivo when introduced into a eukaryotic cell. The set of siRNA fragments comprises **double-stranded** **RNA** of about 15-30 nucleotides that bind specifically to mRNA to initiate cleavage of the mRNA.

USE - The methods are useful in silencing gene expression in mammalian cells. These may be used for generating double-stranded RNAs suitable for silencing of any gene in a rapid, cost-effective and reliable manner. (119 pages)

L19 ANSWER 2 OF 7 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2004-05932 BIOTECHDS
TITLE: New nucleic acid constructs having DNA transcribed into RNA and forms at least one **double-stranded** **RNA** molecule, useful in the field of plant genetics, particularly in providing agents capable of gene-specific silencing;
gene-specific silencing for use in transgenic plant generation
AUTHOR: FILLATTI J J
PATENT ASSIGNEE: MONSANTO TECHNOLOGY LLC

PATENT INFO: WO 2004001000 31 Dec 2003
APPLICATION INFO: WO 2003-US19437 20 Jun 2003
PRIORITY INFO: US 2002-390186 21 Jun 2002; US 2002-390186 21 Jun 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-082485 [08]
AN 2004-05932 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A nucleic acid construct (I) comprises DNA which is transcribed into RNA that forms at least one **double-stranded RNA** molecule, wherein one strand of the double-stranded molecule is coded by a **portion** of the DNA which is at least 90% identical to at least one transcribed intron of a gene.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a transformed cell or organism having (I) in its genome; (2) a transformed plant having (I) in its genome; and (3) **reducing expression** of a protein by a **target** gene in a mammal or plant, comprising introducing (I) into a cell or organism, or into a plant genome.

BIOTECHNOLOGY - Preferred Construct: One strand of the double-stranded molecule of (I) is coded by a **portion** of the DNA which is at least 98% or 100% identical to at least one transcribed intron of a gene. (I) further comprises in series one strand of an intron, a spliceable intron, and the **complement** of the intron, wherein the spliceable intron provides a hairpin structure, and wherein the intron and the **complement** of he intron can hybridize to each other. The transcribed introns are in FAD2 or FAD3 genes. (I) also comprises DNA which is transcribed into RNA that forms at least one **double-stranded RNA** molecule wherein one strand of the molecule is coded by a **portion** of the DNA which is at least 90% identical to at least two transcribed introns. (I) additionally comprises DNA which is transcribed into RNA that forms two or more **double-stranded RNA** molecules.
Preferred Plant: The expression of a protein encoded by the FAD2 or FAD3 gene is **reduced**, substantially **reduced** or effectively eliminated.Preferred Method: The target gene in the method of (3) encodes a protein in an insect or nematode which is a pest to a plant, and wherein the method further comprises introducing into the genome of the plant a nucleic acid construct comprising DNA which is transcribed into RNA that forms at least one **double-stranded RNA** molecule which is effective for **reducing expression** of the **target** gene when the insect or nematode ingests cells from the plant.

USE - The methods and compositions of the present invention are useful in the field of plant genetics, particularly in providing agents capable of gene-specific silencing.

EXAMPLE - Experimental protocols are described but no results given. (81 pages)

L19 ANSWER 3 OF 7 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-21525 BIOTECHDS
TITLE: Novel isolated DNA molecule comprising expressible template nucleotide sequence encoding an intermediate small interfering RNA molecule which mediates RNA interference of target RNA;
antisense hybridization for gene knockout transgenic animal construction and gene silencing study
AUTHOR: WANG J
PATENT ASSIGNEE: ALLELE BIOTECHNOLOGY and PHARM INC
PATENT INFO: WO 2003057840 17 Jul 2003
APPLICATION INFO: WO 2002-US41642 26 Dec 2002
PRIORITY INFO: US 2002-217564 12 Aug 2002; US 2001-343697 27 Dec 2001
DOCUMENT TYPE: Patent
LANGUAGE: English

OTHER SOURCE: WPI: 2003-598368 [56]
AN 2003-21525 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Isolated DNA molecule (I) comprising expressible template nucleotide sequence of at least 16 nucleotides encoding an intermediate small interfering RNA molecule (siRNA) which mediates RNA interference of target RNA, is new.

DETAILED DESCRIPTION - An isolated DNA molecule (I) comprising an expressible template nucleotide sequence of at least 16 nucleotides encoding an intermediate small interfering RNA molecule (siRNA) which mediates RNA interference of target RNA and comprises: (a) 5' **portion**, which comprises at least 15 nucleotides **complementary** to a sense strand of the target RNA, and a 3' terminal **portion** which comprises 1-5 nucleotides that are not **complementary** to the sense strand of the target RNA, where the siRNA selectively hybridizes to the sense strand of the target RNA; or (b) a 5' **portion**, which comprises at least 15 nucleotides **complementary** to an antisense strand of the target RNA, and a 3' terminal **portion**, comprising about 1-5 nucleotides that are not **complementary** to the antisense strand of the target RNA, where the siRNA selectively hybridizes to the antisense strand of the target RNA. INDEPENDENT CLAIMS are also included for the following: (1) a vector (II) comprising (I); (2) a cell (III) which contains (I); (3) several isolated DNA molecules comprising at least two of (I); (4) a DNA-mediated silencing of gene (DMSG) cassette (IV) comprising (I) operatively linked to at least one heterologous nucleotide sequence; (5) a vector (V) comprising (IV); (6) a cell (VI) which contains (IV); (7) several DMSG cassettes comprising at least two of (IV); (8) a kit (VII) comprising (I) or (IV); (9) an isolated modified U6 gene enhancer; (10) a non-human transgenic organism (VIII), comprising (IV); (11) several isolated DNA molecules (IX), where each DNA molecule is immobilized on a solid support, and comprises an expressible template nucleotide sequence of at least 16 nucleotides encoding an siRNA, which mediates RNA interference of a target RNA; (12) a kit, which contains (IX); (13) introducing (IV) into a cell; and (14) a DMSG cassette which **reduces** or **inhibits** expression of the gene in a cell (e.g., cancer cell) containing the DMSG cassette.

WIDER DISCLOSURE - The following are disclosed: (a) producing a genetically modified organism where one or more (IV) is inherited from one generation through the next generation by duplication of chromosomes; (b) a gene identified by sequencing of a template sequence on a DMSG cassette that when introduced into cells, produces a specific phenotype; and (c) mapping the networking relationship of cellular or tissue functions of various genes.

BIOTECHNOLOGY - Preferred Molecule: The intermediate siRNA is 16-30, 20-25, more preferably 21 nucleotides in length. The 3' terminal **portion** of the intermediate siRNA is 2-4, or is 2-3 nucleotides in length. (I) is a double-stranded DNA molecule, where one strand of the double-stranded DNA molecule encodes a first intermediate siRNA, which is **complementary** to the sense strand of the target RNA, and where a second strand of the double-stranded DNA molecule encodes a second intermediate siRNA, which is **complementary** to the antisense strand of the target RNA. (I) is a linear DNA molecule having a first end and a second end. Preferred Cassette: In (IV), the heterologous nucleotide sequence comprises a restriction endonuclease recognition site, a recombinase recognition site, or its combination. The heterologous nucleotide sequence comprises a transcriptional regulatory element such as promoter, enhancer, terminator, or its combination. Preferably, the element comprises an RNA polymerase III transcriptional regulatory element e.g., human U6 gene RNA polymerase III transcriptional regulatory element. (IV) comprises in operative linkage, a promoter, (I) and a terminator. The expressible template nucleotide sequence encodes a first intermediate siRNA and where the heterologous nucleotide sequence comprises a second expressible intermediate siRNA, where the 5'

portion of the second intermediate siRNA is **complementary** to the 5' **portion** of the first intermediate siRNA, and thus upon expression the 5' **portion** of the first intermediate siRNA selectively hybridizes to the 5' **portion** of the second intermediate siRNA, thereby forming a hairpin structure. The cassette further comprises at least one RNA polymerase III transcriptional regulatory element, and one human U6 transcriptional regulatory element. The cassette is a linear or circular expression cassette and further comprises a detectable label chosen from a fluorescent label, a radionuclide, an enzyme, a paramagnetic label, a bioluminescent label or a chemiluminescent label. The cassette further comprises a targeting moiety e.g., a polynucleotide, a peptide, a peptidomimetic or a small organic molecule. The targeting moiety comprises a ligand for a cellular receptor, a receptor for a cellular ligand or an antibody. Most preferably, (IV) comprises in operative linkage, an RNA polymerase III (pol III) promoter, an expressible template nucleotide sequence, and at least one pol III terminator, where the expressible template nucleotide sequence is heterologous with respect to the pol III promoter. The pol III promoter or pol III terminator comprises a mammalian (human or mouse) U6 gene pol III promoter or pol III terminator. The cassette further comprises a operatively linked enhancer. The cassette is a double-stranded DNA molecule, where one strand of the double-stranded DNA molecule encodes the first intermediate siRNA, which is **complementary** to a sense strand of the target RNA, where a second strand of the double-stranded DNA molecule encodes a second intermediate siRNA, which is **complementary** to the antisense strand of the target RNA, and where first intermediate siRNA and second siRNA selectively hybridize to from a double-stranded siRNA. The **double -stranded RNA** comprises a 3' overhang of 1-4 nucleotides at each terminus. Preferred Method: Introducing (IV) into a cell involves contacting (IV), which is immobilized on a solid support, with the cell under conditions sufficient for the DMSG cassette to enter the cell, thus introducing (IV) into the cell. The cassette further comprises operatively linked transmembrane domain peptide, which is a substrate for an intramembrane cleaving protease, where the DMSG cassette is immobilized to the solid support through a transmembrane domain peptide which comprises a peptide of a beta-amyloid precursor protein, a Drosophila sevenless protein or its combination, a Drosophila torso protein or its mammalian homolog, a Drosophila delta protein or its mammalian homolog, or a human glycophorin-A protein. The transmembrane domain peptide is a substrate for a presenilin. (IV) further comprises an operatively linked protein transduction domain, which comprises a human immunodeficiency virus TAT domain, a Drosophila Antennapaedia homeodomain, a herpes simplex virus VP22 transduction domain, or a fibroblast growth factor transduction domain. The DSMG cassette comprises several DMSG cassettes each of which is immobilized to the solid support, and are positioned in an array, e.g. DNA array. Upon expression of the expressible nucleotide sequence of DMSG cassette in a cell containing the DMSG cassette expression of the gene in the cell is **reduced** or **inhibited**. The **reduced** or **inhibited** expression of the gene is detected by detecting a phenotypic change of the cell, where the gene encodes the transcription factor, a growth factor, a growth factor receptor, protein kinase or a G protein. Optionally, the gene is expressed in the cell exhibiting a pathological condition, but not in a corresponding cell that does not exhibit a pathologic conditions. The cell exhibiting pathologic condition is a cancer cell.

ACTIVITY - None given.

MECHANISM OF ACTION - **Reduces** or **inhibits** expression of the gene; Gene silencing inducer; Mediator of RNA interference of target RNA. Oligonucleotides for DMSG cassettes specific for a nucleotide sequence encoding green fluorescent protein (GFP) were synthesized. The expression cassette (from 5' to 3') consists of an enhancer region, the distal sequence element (DSE, -79 to -72 in

reference to the transcription start site on the cassette) for pol III gene U6 (human), a proximal sequence element (PSE, -66 TO -47) for pol III human gene U6, a TATA box (-31 to -26), followed by the template sequence (21 nucleotides), a terminator of the U6 gene, and an artificial terminator (TM or Term). Forty-eight hours after transfection, cells were analyzed under an inverted fluorescence microscope. To evaluate whether the transcripts generated from the DMSG cassettes against GFP expression could specifically block gene expression, pEGFP plasmid was used as a reporter in a co-transfection study. GFP expression was **reduced** in cells transfected with GFP siRNA. The expression of GFP in these cells was monitored for over 2 weeks and the gene silencing effects by DMSG was persistent during this time period.

USE - (I) is useful for assessing the function of a gene in a test cell, which involves introducing at least one (IV) into the test cell, and observing a phenotype of the test cell upon expression of the siRNA encoded by the DMSG cassette, whereby a comparison of the phenotype of the test cell as compared to a control cell is indicative of a function of the target gene, thus assessing the function of the gene in the test cell. (I) is also useful for determining whether an agent effects a specific gene in a test cell, which involves expressing an siRNA comprising the intermediate siRNA encoded by at least one (IV) in the test cell, where the intermediate siRNA comprises a 5' **portion complementary** to an RNA molecule encoded by the specific gene in the test cell, contacting the test cell and a control cell with the agent, and comparing a phenotype of the test cell with that of the control cell, thus assessing whether the agent effects the specific gene in the test cell. (IV) is useful for mediating RNA interference of a target RNA in a cell, which involves introducing at least one (IV) into the cell, whereby expression of an siRNA comprising the intermediate siRNA encoded by the DMSG cassette triggers degradation of the target RNA, thus mediating RNA interference in the cell. (IV) is also useful for knocking down **expression** of a **target** gene in a sample, which involves contacting the sample with at least one (IV), where expression of an siRNA comprising the intermediate siRNA encoded by the DMSG cassette triggers degradation of a target RNA molecule encoded by the target gene, thus knocking down, either partially or completely, **expression** of the **target** gene in the sample. (IV) is also useful for ameliorating an RNA mediated disorder in an individual by inducing RNA against the target RNA mediating the disorder, which involves contacting cells of the individual that exhibit the RNA-mediated disorder with at least one (IV), where expression of an siRNA comprising one or more intermediate siRNA molecules encoded by the template nucleotide sequence of DMSG cassette can mediate RNAi against the target RNA. (IV) (further comprising at least one human U6 gene transcriptional regulatory element) is useful in tracking, among a population of cells, a specific cell or specific group of cells subject to DNA mediated gene silencing, which involves introducing at least (IV) into the specific cell or into each cell of the specific group of cells, and detecting the detectable label, thus tracking, among the population of cells, the specific cell or specific group of cells subject to DNA mediated gene silencing. (IV) comprising the regulatory element as described above is also useful for identifying a cell subject to DNA mediated gene silencing which involves contacting at least one cell with at least one (IV) under conditions sufficient for introduction of a DMSG cassette into a cell, and detecting a detectable label of the at least one (IV) in the cell (all claimed).

ADMINISTRATION - Composition comprising (IV) is administered orally or parenterally, such as intravenously, intramuscularly, subcutaneously, intraperitoneally, or by passive or facilitated absorption through the skin. No specific clinical dosages are given.

ADVANTAGE - RNAi can be performed without the need to manipulate RNA molecules outside of a target cell. RNAi can be induced for transient gene knock down, as well as for permanent gene knock down because DNA molecules encoding the RNAi can be inserted into the chromosome of the

target cell. Gene-specific silencing can be restricted to one or few cell types, or to a particular time, without causing global shut down of the genes in other cells of an organism. (132 pages)

L19 ANSWER 4 OF 7 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2004-02441 BIOTECHDS
TITLE: New nucleic acids, useful for **inhibiting** the synthesis of a target protein in a eukaryotic cell, or for treating various diseases by **inhibiting** the expression of abnormal or mutated proteins, e.g. leukemia, viral or bacterial infection;
target protein **inhibition** and viru vector **expression** in host cell for use in disease gene therapy
AUTHOR: SHI Y; SUI G
PATENT ASSIGNEE: SHI Y; SUI G
PATENT INFO: US 2003180756 25 Sep 2003
APPLICATION INFO: US 2002-301516 21 Nov 2002
PRIORITY INFO: US 2002-301516 21 Nov 2002; US 2002-366478 21 Mar 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-852231 [79]
AN 2004-02441 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A new nucleic acid comprising in a 5'-3' order: an RNA polymerase promoter sequence; a first target sequence that is essentially **complementary** to a sequence of a target nucleic acid or its **complement**; a spacer sequence; a second target sequence that is essentially **complementary** to the first target sequence; and an RNA polymerase termination signal, where an RNA transcribed from the nucleic acid can **inhibit expression** of the **target** gene.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) an RNA comprising the following nucleotide sequences in a 5'-3' order: a first target sequence of about 19-25 nucleotides, which is at least about 95% identical to a **portion** of a nucleotide sequence of a target nucleic acid or its **complement**, a spacer sequence of about 5-10 nucleotides, a second target sequence of about 19-25 nucleotides that is essentially **complementary** to the first target sequence, and at least a **portion** of an RNA polymerase termination signal, where the RNA **inhibits expression** of a **target** gene comprising a sequence that is essentially **complementary** to the first or the second target sequence; (2) a cell comprising the nucleic acid cited above; (3) a method for preparing a nucleic acid for **inhibiting** the synthesis of a target protein in a eukaryotic cell; (4) a method for producing RNA molecules that **inhibit expression** of a **target** nucleic acid in a eukaryotic cell; (5) methods for **inhibiting** the synthesis of a target protein in a eukaryotic cell or in a cell of a subject; and (6) a kit for **inhibiting** the synthesis of a target protein in a cell, comprising the nucleic acid cited above and at least one reagent for introducing the nucleic acid into a cell.

BIOTECHNOLOGY - Preferred Nucleic Acid: The RNA transcribed from the nucleic acid forms a hairpin structure. The polymerase is preferably RNA polymerase III (Pol III) and the polymerase termination signal comprises a number of thymidines sufficient for arresting Pol III activity. The first target sequence is at least about 95% identical to a nucleotide sequence of the target nucleic acid or its **complement**. The first target sequence is perfectly **complementary** to a sequence of a target nucleic acid or its **complement**. The target nucleic acid is a target gene. The first and the second target sequences comprise about 15-30, preferably 19-25 nucleotides. The first target sequence comprises a **portion** of the coding sequence of the target

nucleic acid or its **complement**. The first and the second target sequences differ in at most 2 nucleotides, and are perfectly **complementary**. The number of thymidines sufficient for arresting Pol III activity is 4 or 5 thymidines. The spacer sequence consists of about 3-15 or 5-10, preferably 6 nucleotides. The spacer sequence comprises a palindromic sequence, which is AACGTT. The Pol III promoter comprises a U6 promoter. The Pol III promoter comprises from about nucleotide -315 to about nucleotide +1 of the mouse U6 promoter having a fully defined sequence of 316 bp given in the specification. The nucleic acid is DNA, and is in a plasmid or in an expression vector. The expression vector is a eukaryotic expression vector, which is a mammalian expression vector or a viral vector. Preferably, the viral vector is an adenoviral vector. In the nucleic acid cited above, the polymerase is a Pol III, the first target sequence is essentially **complementary** to a sequence of a target nucleic acid or its **complement**, the first and the second target sequences consist of about 19-23 nucleotides and are perfectly **complementary** to each other, the spacer sequence consists of about 6 nucleotides, and the RNA polymerase termination signal consists of 4 or 5 thymidines. The nucleic acid preferably comprises the following nucleotide sequences in a 5'-3' order: a Pol III promoter sequence, a first restriction enzyme recognition sequence, a spacer sequence, a second restriction enzyme recognition sequence, and a number of thymidines sufficient for arresting Pol III activity, where an RNA molecule transcribed from the nucleic acid in which a first and a second target sequences are inserted in the first and second restriction enzyme recognition site, respectively, **inhibits expression** of a **target** gene comprising a sequence that is essentially **complementary** to the first or the second target sequence. The nucleic acid further comprises at least one additional restriction enzyme recognition sequence between the Pol III promoter and the first restriction enzyme recognition sequence, or between the second restriction enzyme recognition sequence and the thymidines sufficient for arresting Pol III activity. Preferred RNA: The RNA forms a hairpin structure. The first and the second target sequences consist of about 19-23 nucleotides and are perfectly **complementary** to each other, the first target sequence is perfectly **complementary** to a sequence of the target nucleic acid or its **complement**, and the polymerase termination signal consists of 4 or 5 uridines. Preferred Cell: The cell is a eukaryotic cell, preferably a mammalian cell. The cell is an isolated cell. Preferred Method: Preparing a nucleic acid for **inhibiting** the synthesis of a target protein in a eukaryotic cell comprises providing the nucleic acid cited above, and introducing into the first restriction recognition sequence a first oligonucleotide of about 15-30 nucleotides comprising a sequence that is essentially **complementary** to a sequence of the target nucleic acid. The method further comprises introducing into the second restriction recognition sequence a second oligonucleotide of about 15-30 nucleotides that is essentially **complementary** to the sequence of the first oligonucleotide. The first oligonucleotide comprises about 20-23 consecutive nucleotides of the target nucleic acid or its **complement**. The method further comprises introducing into the second restriction recognition sequence a second oligonucleotide comprising a nucleotide sequence that is perfectly **complementary** to the sequence of the first oligonucleotide. Producing RNA molecules that **inhibit expression** of a **target** nucleic acid in a eukaryotic cell comprises introducing into a eukaryotic cell the nucleic acid above, where the first target sequence is essentially **complementary** to a sequence of the target nucleic acid or its **complement**, such that the nucleic acid is transcribed in the eukaryotic cell and produces RNA molecules that **inhibit expression** of a **target** nucleic acid. The first target sequence is perfectly **complementary** to a sequence of the target nucleic acid and the first and the second target sequences consist of about 19-25 nucleotides and arc perfectly

complementary to each other. **Inhibiting** the synthesis of a target protein in a eukaryotic cell comprises introducing into a target cell the nucleic acid above, where the first target sequence is essentially or perfectly **complementary** to a sequence of the nucleic acid encoding the target protein or its **complement**, such that the nucleic acid is transcribed in the target cell and **inhibits** the synthesis of the target protein. The cell is an isolated cell. **Inhibiting** the synthesis of a target protein in a cell of a subject comprises introducing into the cell of the subject the nucleic acid above, where the first target sequence is essentially or perfectly **complementary** to a sequence of the gene encoding the target protein or its **complement**, such that the nucleic acid is transcribed in the target cell and **inhibits** the synthesis of the target protein. The method comprises first obtaining the cell from a subject, introducing the nucleic acid into the cell ex vivo, and administering the cell to the subject.

ACTIVITY - Cytostatic; Hemostatic; Virucide; Antibacterial; Neuroprotective; Nootropic; Anticonvulsant; Antiparkinsonian. No biological data given.

MECHANISM OF ACTION - Gene Therapy.

USE - The nucleic acids and methods are useful for suppressing gene expression in cells, or **inhibiting** the synthesis of a target protein in a eukaryotic cell or in a cell of a subject. The nucleic acids can be used for treating various diseases by **inhibiting** the expression of abnormal or mutated proteins, e.g. cancers such as leukemia, hemophilia, viral or bacterial infections, or neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis.

ADMINISTRATION - Administration may be oral or parenteral, including intravenous, intramuscular, intraperitoneal, subcutaneous, rectal and topical. No dosage given.

EXAMPLE - DNA fragments that acted as templates for the synthesis of small RNAs were inserted under the control of the mouse U6 promoter that directs the synthesis of a polymerase III (Pol III)-specific RNA transcript. The resulting RNA was composed of two identical 21-nucleotide sequence motifs in an inverted orientation separated by a 6-bp spacer of non-homologous sequences. Five Ts that function as a termination signal for Pol III were added at the 3' end of the repeat. This RNA was predicted to fold back to form a hairpin **double stranded RNA** with a 3' overhang of several Ts. Although the exact structure of this small RNA is unknown, it robustly **inhibited** gene expression in vivo. (38 pages)

L19 ANSWER 5 OF 7 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2004-04620 BIOTECHDS
TITLE: New SID-1 protein and nucleic acid sequences, useful for silencing gene expression, or for **reducing** the **expression** of a **target** gene in a cell, a population of cells, or an animal; involving vector-mediated gene transfer and expression in host cell for use in transgenic animal model construction and drug screening
AUTHOR: HUNTER C P; WINSTON W M; MOLDOWITCH C
PATENT ASSIGNEE: HUNTER C P; WINSTON W M; MOLDOWITCH C
PATENT INFO: US 2003167490 4 Sep 2003
APPLICATION INFO: US 2002-304930 26 Nov 2002
PRIORITY INFO: US 2002-304930 26 Nov 2002; US 2001-333325 26 Nov 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-898128 [82]
AN 2004-04620 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An isolated nucleic acid (I) encoding a SID-1 protein, is new.

DETAILED DESCRIPTION - (I) comprises: (a) a nucleotide sequence of

Inhibition of undesirable gene expression with **double-stranded RNA**

INVENTOR(S): Kreutzer, Roland; Limmer, Stephan
PATENT ASSIGNEE(S): Germany
SOURCE: PCT Int. Appl., 57 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000044895	A1	20000803	WO 2000-DE244	20000129
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
DE 19956568	A1	20000817	DE 1999-19956568	19991124
CA 2359180	AA	20000803	CA 2000-2359180	20000129
EP 1144623	A1	20011017	EP 2000-910510	20000129
EP 1144623	B1	20020828		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
EP 1214945	A2	20020619	EP 2002-3683	20000129
EP 1214945	A3	20020904		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL				
AT 222953	E	20020915	AT 2000-910510	20000129
JP 2003502012	T2	20030121	JP 2000-596137	20000129
ES 2182791	T3	20030316	ES 2000-910510	20000129
DE 20023125	U1	20030618	DE 2000-20023125	20000129
ZA 2001005909	A	20020724	ZA 2001-5909	20010718
US 2004053875	A1	20040318	US 2003-382768	20030306
US 2004072779	A1	20040415	US 2003-382395	20030306
US 2004102408	A1	20040527	US 2003-383099	20030306

PRIORITY APPLN. INFO.:
DE 1999-19903713 A 19990130
DE 1999-19956568 A 19991124
EP 2000-910510 A3 20000129
WO 2000-DE244 W 20000129
US 2001-889802 A3 20010917

AB The invention relates to a medicament containing at least one double-stranded oligoribonucleotide (dsRNA) designed to **inhibit** the **expression** of a **target** gene. According to the invention, one strand of the dsRNA is at least in part complementary to the target gene. Thus, **inhibition** of model gene expression both in vitro and in vivo (3T3 cells) by **double-stranded RNA** was demonstrated. A 21-nucleotide oligoribonucleotide, to which the complementary oligoribonucleotide was covalently attached via a linker, was shown to be an effective **inhibitor**.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=>

Gene function analysis by RNA interference using **double-stranded RNA** and reporter gene

INVENTOR(S): Saigou, Kaoru; Tei, Kumiko
PATENT ASSIGNEE(S): Mitsubishi Chemical Corp., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 12 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2002306183	A2	20021022	JP 2001-166101	20010601

PRIORITY APPLN. INFO.: JP 2000-165414 A 20000602

AB A method and kit for anal. of gene function by introducing **double-stranded RNA** (dsRNA) **complementary** to the target gene sequence or inverted repeat sequence-containing RNA, into cell culture of vertebrate origin and observing the phenotype change, is disclosed. An RNA library may be introduced. A reporter gene is also introduced, and its expression is measured. The firefly luciferase gene and Renilla reniformis luciferase gene were used as target and reporter, resp. DsRNA introduced into CHO-k1 cells or 293 cells effectively **inhibited** the expression of firefly luciferase gene. Inverted repeat sequence-containing RNA also **inhibited** the **expression** of the **target** gene.

Inhibiting expression of **target** gene, useful e.g. for treating cancer, by infecting cells with sequences encoding sense and antisense RNA homologous with the target;
virus vector-mediated gene transfer, expression in host cell and sense and antisense RNA useful for gene therapy and functional genomics

AUTHOR: CERTA U; LUNDSTROM K
PATENT ASSIGNEE: HOFFMANN LA ROCHE and CO AG F
PATENT INFO: FR 2817265 31 May 2002
APPLICATION INFO: FR 2000-15363 29 Nov 2000
PRIORITY INFO: EP 2000-126113 29 Nov 2000
DOCUMENT TYPE: Patent
LANGUAGE: French
OTHER SOURCE: WPI: 2002-511084 [55]
AN 2002-18608 BIOTECHDS

AB DERWENT ABSTRACT:
NOVELTY - Method for **inhibiting expression** of a **target** gene (I) in cells or tissue by infection with separate viral particles (VP) that contain single-stranded RNA (ssRNA) that express sense and antisense RNA, both containing sequences homologous with a part of (I).

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a kit containing both types of VP that produce **complementary** RNAs so that, in a cell or tissue, they form a **double-stranded RNA** that interferes with expression of (I).

WIDER DISCLOSURE - This also describes cells in which the two ssRNA structures interfere with expression of (I).

BIOTECHNOLOGY - Preferred Particles: These are from alphavirues, preferably Semliki Forest, Sindbis or Venezuelan equine encephalitis viruses, and contain ssRNA cloned in either orientation into the vector for VP. The vector preferably contains the genes for non-structural proteins 1-4, essential for viral replication. The homologous sequences contain at least 50, especially 75-125, bases. Preferred Process: Equal numbers of both types of VP are used, preferably at least 10 per cell. (I) is a eukaryotic, viral or synthetic gene, especially a developmental gene, oncogene, or the gene for a tumor suppressor or enzyme. The cell or tissue may be present in a living organism and **inhibiting** expression of (I) results in a loss of phenotypical function.

ACTIVITY - Cytostatic; Virucide; Anti-HIV. No details of tests for these activities are given.

MECHANISM OF ACTION - **Inhibiting** expression of specific genes.

USE - The method is used for treating or preventing diseases associated with overexpression of particular genes, especially cancer (solid tumors or leukemia), but also virus, e.g. human immune deficiency virus, infection. It can also be used to identify a genetic function in an organism and for analysis of the mechanisms of growth, development, metabolism, resistance to disease and other processes.

ADMINISTRATION - VP are administered by injection. No doses are suggested.

ADVANTAGE - The introduced ss-RNA sequences are efficiently replicated in cells, and the **double-stranded RNA** formed is stable, safe and provides gene-specific **inhibition**.

EXAMPLE - Human embryonic kidney 293 cells (ATCC CRL-1573) were infected (multiplicity of infection not stated) with Semliki Forest virus particles (SFVP) that express a sense RNA homologous with part of the cyclin gene, a similar SFVP but expressing an antisense sequence or both types of SVFP. After 40 hr, growth **inhibition** was assessed by measurement of optical density (OD) at 492 nm. Results were: about 2.1 and about 1.4, compared with 3.8 (maximal proliferation) for a control. When the cells were infected with particles that express two pairs of sense and antisense RNA, both homologous with the cyclin gene, OD was

reduced to about 1.2. (29 pages)

L18 ANSWER 32 OF 37 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:539827 CAPLUS
DOCUMENT NUMBER: 137:104780
TITLE: Method for **inhibiting** the **expression** of a **target** gene
INVENTOR(S): Kreutzer, Roland; Limmer, Stephan; Rost, Sylvia; Hadwiger, Philipp
PATENT ASSIGNEE(S): Ribopharma Ag, Germany
SOURCE: PCT Int. Appl., 203 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 9
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002055693	A2	20020718	WO 2002-EP152	20020109
WO 2002055693	A3	20030717		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
DE 10100586	C1	20020411	DE 2001-10100586	20010109
DE 10160151	A1	20030626	DE 2001-10160151	20011207
EP 1352061	A2	20031015	EP 2002-710786	20020109
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
DE 10230996	A1	20030717	DE 2002-10230996	20020709
DE 10230997	A1	20030717	DE 2002-10230997	20020709
WO 2003033700	A1	20030424	WO 2002-EP11432	20021011
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
WO 2003035868	A1	20030501	WO 2002-EP11968	20021025
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
WO 2003035869	A1	20030501	WO 2002-EP11969	20021025
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,				

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LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,
PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ,
UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD,
RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG,
CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,
PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
NE, SN, TD, TG

WO 2003035870 A1 20030501 WO 2002-EP11970 20021025

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
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RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG,
CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,
PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
NE, SN, TD, TG

WO 2003035082 A1 20030501 WO 2002-EP11971 20021025

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
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RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG,
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PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
NE, SN, TD, TG

WO 2003035083 A1 20030501 WO 2002-EP11972 20021025

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
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RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG,
CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,
PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
NE, SN, TD, TG

WO 2003035876 A1 20030501 WO 2002-EP11973 20021025

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
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RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG,
CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,
PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
NE, SN, TD, TG

US 2004091457 A1 20040513 US 2003-384512 20030307
US 2004121348 A1 20040624 US 2003-384434 20030307
US 2004038921 A1 20040226 US 2003-382634 20030811
US 2004126791 A1 20040701 US 2003-666458 20030919

PRIORITY APPLN. INFO.: DE 2001-10100586 A 20010109
DE 2001-10155280 A 20011026

DE 2001-10158411 A 20011129
DE 2001-10160151 A 20011207
DE 2001-10150187 A 20011012
DE 2001-10163098 A 20011220
WO 2002-EP151 A 20020109
WO 2002-EP152 W 20020109
DE 2002-10230996 A 20020709
DE 2002-10230997 A 20020709
DE 2002-10235620 A 20020802
DE 2002-10235621 A 20020802
WO 2002-EP11432 A2 20021011
WO 2002-EP11968 A2 20021025
WO 2002-EP11970 A2 20021025
WO 2002-EP11971 A2 20021025

AB A method of **inhibiting** the expression of a specific gene using double-stranded oligoribonucleotides (dsRNA) capable of hybridizing with the gene is described. The dsRNA has a double-stranded core that is no more than 49 base-pairs long and has one or two 1-4 nucleotide single-stranded ends. **Inhibition** of expression of green fluorescent protein reporter gene in vitro and in vivo (3T3 and HeLa-S3 cells) by **double-stranded RNA** was demonstrated. A 21-nucleotide oligoribonucleotide, to which the **complementary** oligoribonucleotide was covalently attached via a linker, was shown to be an effective **inhibitor**. **Inhibition** of expression of the genes for epidermal growth factor receptors in the glioblastoma cell line U87MG is demonstrated.

L18 ANSWER 33 OF 37 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:800769 CAPLUS
DOCUMENT NUMBER: 137:321237
TITLE: Gene function analysis by RNA interference using **double-stranded RNA** and reporter gene
INVENTOR(S): Saigou, Kaoru; Tei, Kumiko
PATENT ASSIGNEE(S): Mitsubishi Chemical Corp., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 12 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2002306183	A2	20021022	JP 2001-166101	20010601

PRIORITY APPLN. INFO.: JP 2000-165414 A 20000602

AB A method and kit for anal. of gene function by introducing **double-stranded RNA** (dsRNA) **complementary** to the target gene sequence or inverted repeat sequence-containing RNA, into cell culture of vertebrate origin and observing the phenotype change, is disclosed. An RNA library may be introduced. A reporter gene is also introduced, and its expression is measured. The firefly luciferase gene and Renilla reniformis luciferase gene were used as target and reporter, resp. DsRNA introduced into CHO-k1 cells or 293 cells effectively **inhibited** the expression of firefly luciferase gene. Inverted repeat sequence-containing RNA also **inhibited** the **expression** of the **target** gene.

L18 ANSWER 34 OF 37 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2002:556450 SCISEARCH
THE GENUINE ARTICLE: 565VN
TITLE: Both natural and designed micro RNAs technique can **inhibit** the expression of cognate mRNAs when expressed in human cells

AUTHOR: Zeng Y; Wagner E J; Cullen B R (Reprint)
CORPORATE SOURCE: Duke Univ, Med Ctr, Dept Mol Genet & Microbiol, Durham, NC 27710 USA (Reprint); Duke Univ, Med Ctr, Howard Hughes Med Inst, Durham, NC 27710 USA
COUNTRY OF AUTHOR: USA
SOURCE: MOLECULAR CELL, (JUN 2002) Vol. 9, No. 6, pp. 1327-1333. Publisher: CELL PRESS, 1100 MASSACHUSETTES AVE,, CAMBRIDGE, MA 02138 USA. ISSN: 1097-2765.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 26

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Animal cells have recently been shown to express a range of similar to22 nucleotide noncoding RNAs termed micro RNAs (miRNAs). Here, we show that the human mir-30 miRNA can be excised from irrelevant, endogenously transcribed mRNAs encompassing the predicted 71 nucleotide mir-30 precursor. Expression of the mir-30 miRNA specifically blocked the translation in human cells of an mRNA containing artificial mir-30 target sites. Similarly, designed miRNAs were also excised from transcripts encompassing artificial miRNA precursors and could **inhibit** the **expression** of mRNAs containing a **complementary target** site. These data indicate that novel miRNAs can be readily produced in vivo and can be designed to specifically inactivate the **expression** of selected **target** genes in human cells.

L18 ANSWER 35 OF 37 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2002078962 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11804566
TITLE: Novel genomic cDNA hybrids produce effective RNA interference in adult Drosophila.
AUTHOR: Kalidas Savitha; Smith Dean P
CORPORATE SOURCE: Department of Pharmacology and Center for Basic Neuroscience, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390, USA.
CONTRACT NUMBER: DC02539 (NIDCD)
SOURCE: Neuron, (2002 Jan 17) 33 (2) 177-84. Journal code: 8809320. ISSN: 0896-6273.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200202
ENTRY DATE: Entered STN: 20020128
Last Updated on STN: 20020212
Entered Medline: 20020211

AB Drosophila melanogaster has been a premier genetic model system for nearly 100 years, yet lacks a simple method to disrupt gene expression. Here, we show genomic cDNA fusions predicted to form **double-stranded RNA** (dsRNA) following splicing, effectively silencing **expression** of **target** genes in adult transgenic animals. We targeted three Drosophila genes: lush, white, and dGq(alpha). In each case, **target** gene **expression** is dramatically **reduced**, and the white RNAi phenotype is indistinguishable from a deletion mutant. This technique efficiently targets genes expressed in neurons, a tissue refractory to RNAi in C. elegans. These results demonstrate a simple strategy to knock out gene function in specific cells in living adult Drosophila that can be applied to define the biological function of hundreds of orphan genes and open reading frames.

L18 ANSWER 36 OF 37 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:535274 CAPLUS
DOCUMENT NUMBER: 133:145885

hairpin RNAs

AUTHOR(S): Smith, Neil A.; Singh, Surinder P.; Wang, Ming-Bo; Stoutjesdijk, Peter A.; Green, Allan G.; Waterhouse, Peter M.
CORPORATE SOURCE: CSIRO Plant Industry, Canberra, ACT 2601, Australia
SOURCE: Nature (London) (2000), 407(6802), 319-320
CODEN: NATUAS; ISSN: 0028-0836
PUBLISHER: Nature Publishing Group
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Post-transcriptional gene silencing (PTGS), a sequence-specific RNA degradation mechanism inherent in many life forms, can be induced in plants by transforming them with either antisense or co-suppression constructs, but typically this results in only a small proportion of silenced individuals. Here we show that gene constructs encoding intron-spliced RNA with a hairpin structure can induce PTGS with almost 100% efficiency when directed against viruses or endogenous genes. Using principles we developed for silencing constructs that express **double-stranded RNA** and inverted-repeat RNA, we made a construct encoding a single self **complementary** hairpin RNA (hpRNA) of the Niaprotease (Pro) gene sequence of potato virus Y (PVY). The construct contains sense and antisense Pro sequences flanking a nucleotide spacer fragment derived from uidaA (GUS) gene. About 60% of the plants that are transformed with the constructs were immune to the virus. In the next experiment, we replaced the spacer with an intron sequence, which is spliced out during pre-mRNA processing to produce loopless hpRNA. As a control, the intron sequence was inserted in the reverse, non-splicing, orientation. When transformed into tobacco, 22 of 34 (65%) reverse-intron plants were immune, a similar frequency to plants transformed with the GUS spacer construct. Amazingly, 22 of 23 plants transformed with the construct containing the functional intron were immune to the virus. This same enhancement was observed when hpRNA constructs against the endogenous .apprch.12-desaturase (Fad2) gene of Arabidopsis, in which 100% (30/30) of plants transformed with the intron construct showed silencing of the gene. The process of intron excision from the construct by spliceosome might help to align the **complementary** arms to the hairpin in an environment favoring RNA hybridization, promoting the formation of a duplex. Alternatively, splicing may transiently increase the amount of hairpin RNA by facilitating, or retarding, the hairpin's passage from the nucleus, or by creating a smaller, **less** nuclease-sensitive loop.

133:145885
TITLE: **Inhibition** of undesirable gene expression with **double-stranded RNA**
INVENTOR(S): Kreutzer, Roland; Limmer, Stephan
PATENT ASSIGNEE(S): Germany
SOURCE: PCT Int. Appl., 57 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000044895	A1	20000803	WO 2000-DE244	20000129
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
DE 19956568	A1	20000817	DE 1999-19956568	19991124
CA 2359180	AA	20000803	CA 2000-2359180	20000129
EP 1144623	A1	20011017	EP 2000-910510	20000129
EP 1144623	B1	20020828		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
EP 1214945	A2	20020619	EP 2002-3683	20000129
EP 1214945	A3	20020904		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL				
AT 222953	E	20020915	AT 2000-910510	20000129
JP 2003502012	T2	20030121	JP 2000-596137	20000129
ES 2182791	T3	20030316	ES 2000-910510	20000129
DE 20023125	U1	20030618	DE 2000-20023125	20000129
ZA 2001005909	A	20020724	ZA 2001-5909	20010718
US 2004053875	A1	20040318	US 2003-382768	20030306
US 2004072779	A1	20040415	US 2003-382395	20030306
US 2004102408	A1	20040527	US 2003-383099	20030306

PRIORITY APPLN. INFO.:
DE 1999-19903713 A 19990130
DE 1999-19956568 A 19991124
EP 2000-910510 A3 20000129
WO 2000-DE244 W 20000129
US 2001-889802 A3 20010917

AB The invention relates to a medicament containing at least one double-stranded oligoribonucleotide (dsRNA) designed to **inhibit** the **expression** of a **target** gene. According to the invention, one strand of the dsRNA is at least in part **complementary** to the target gene. Thus, **inhibition** of model gene expression both in vitro and in vivo (3T3 cells) by **double-stranded RNA** was demonstrated. A 21-nucleotide oligoribonucleotide, to which the **complementary** oligoribonucleotide was covalently attached via a linker, was shown to be an effective **inhibitor**.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 37 OF 37 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:718761 CAPLUS
DOCUMENT NUMBER: 134:203186
TITLE: Gene expression: Total silencing by intron-spliced

Identification of gene function by introducing it into a verterbrate **cell** and observing changes in the **cell**;
ds RNA transfer and expression in host **cell** for gene silencing study

PATENT ASSIGNEE: MITSUBISHI CHEM CORP
PATENT INFO: JP 2002306183 22 Oct 2002
APPLICATION INFO: JP 2001-166101 1 Jun 2001
PRIORITY INFO: JP 2000-165414 2 Jun 2000; JP 2000-165414 2 Jun 2000
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 2003-397234 [38]
AN 2003-15560 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Identifying the function of a target gene in which an RNA having a substantially same sequence as the base sequence of said target gene at least part of which is double-stranded or an RNA having a reverse direction repeating sequence of said sequence is introduced to a culture **cell** derived from a vertebrate and cultured and the change in character appeared in said **cell** is analyzed, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) preparation of a **cell** having a desired character in which the above RNA is introduced to a culture **cell** derived from a vertebrate (2) the **cell** prepared by the method of (1); and (3) screening a **cell** in which an RNA library at least part of which is double-stranded or an RNA library having reverse direction repeating sequence of said sequence or a DNA library producing said RNA is introduced to a culture **cell** derived from a vertebrate and cultured and a desired one among the characters which appeared in the **cell** as the result is selected.

USE - The method is used for identifying the function of a **target** gene.

EXAMPLE - **Expression** of a gene was **inhibited** by a **double-stranded RNA** introduced in CHO cells. (12 pages)

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131:54712
TITLE: **Inhibition** of gene expression via injection of **double-stranded RNA**
INVENTOR(S): Fire, Andrew; Xu, Siqun; Montgomery, Mary K.; Kostas, Stephen A.; Timmons, Lisa; Tabara, Hiroaki; Driver, Samuel E.; Mello, Craig C.
PATENT ASSIGNEE(S): Carnegie Institution of Washington, USA
SOURCE: PCT Int. Appl., 54 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9932619	A1	19990701	WO 1998-US27233	19981221
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6506559	B1	20030114	US 1998-215257	19981218
CA 2311999	AA	19990701	CA 1998-2311999	19981221
AU 9919380	A1	19990712	AU 1999-19380	19981221
AU 743798	B2	20020207		
EP 1042462	A1	20001011	EP 1998-964202	19981221
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002516062	T2	20020604	JP 2000-525538	19981221
US 2003051263	A1	20030313	US 2002-283190	20021030
US 2003056235	A1	20030320	US 2002-282996	20021030
US 2003055020	A1	20030320	US 2002-283267	20021030

PRIORITY APPLN. INFO.:			
US 1997-68562P	P	19971223	
US 1998-215257	A	19981218	
WO 1998-US27233	W	19981221	

AB The invention provides a process for introducing RNA into a living **cell** to **inhibit expression** of a **target** gene in that **cell**, whereby the RNA is **double-stranded RNA** (dsRNA) and **inhibition** is sequence-specific in that the nucleotide sequences of the duplex region of the RNA and of a portion of the target gene are identical. The invention has been used to **inhibit** expression of 18 different genes from C. elegans, including unc-22, unc-54, fem-1, and hlh-1. Antisense interference, triple-strand interference, and co-suppression are known methods of gene **inhibition**, but the present invention offers advantages over these, including the ease of introducing **double-stranded RNA** (dsRNA) into cells, the low concentration of RNA which can be used, the stability of dsRNA, and the effectiveness of the **inhibition**. Unlike other methods, this invention does not suffer from being limited to a particular set of target genes, a particular portion of the target gene's nucleotide sequence, or a particular transgene or viral delivery method.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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at least 10, 12, 14, 16 or 18 consecutive nucleotides of a fully defined sequence of 2672 bp (N1) given in the specification; (b) a nucleotide sequence encoding a SID-1 protein, or at least a transmembrane or an extracellular domain of a SID-1 protein; (c) a sequence **complementary** to the sequence of (A) or (B); (d) a nucleic acid encoding a polypeptide having at least 80% amino acid sequence identity with a SID-1 protein, or at least a transmembrane or an extracellular domain of a SID-1 protein; (e) a nucleic acid encoding a polypeptide having at least 80% amino acid sequence identity with a SID-1 protein and having SID-1 activity in a cell capable of expressing SID-1 activity; (f) a nucleotide sequence that hybridizes to at least a **portion** of the sequence of N1 under conditions including a wash step of 1.0xSSC at 65degreesC; (g) a nucleotide sequence encoding a polypeptide having SID-1 activity, where the nucleic acid hybridizes to at least a **portion** of the sequence of N1 under conditions including a wash step of 1.0xSSC at 65degreesC, and a heterologous regulatory region operably joined to the sequence such that the sequence is expressed; or (h) a nucleotide sequence encoding a polypeptide having at least 80% amino acid sequence identity with a fully defined sequence of 776 amino acids (P1) given in the specification, and a heterologous regulatory region operably joined to the sequence such that the sequence is expressed. INDEPENDENT CLAIMS are included for the following: (1) a vector comprising (I), a genetic construct capable of expressing (I), or (I) operably joined to a reporter gene; (2) a cell transformed with (I) or with a genetic construct capable of expressing (I); (3) a non-human transgenic animal, where a genetic construct has introduced a genetic modification into a genome of the animal or its ancestor, and where the modification consists of insertion of a nucleic acid encoding at least a fragment of a SID-1 protein, inactivation of an endogenous SID-1 protein, or insertion by homologous recombination of a reporter gene operably linked to SID-1 regulatory elements; (4) a substantially pure protein preparation comprising: (a) a SID-1 protein, or at least a transmembrane or an extracellular domain of a SID-1 protein; (b) a polypeptide having at least 80% amino acid sequence identity with a SID-1 protein, or at least a transmembrane or an extracellular domain of a SID-1 protein; or (c) a polypeptide having at least 80% amino acid sequence identity with a SID-1 protein and having SID-1 activity in a cell capable of expressing SID-1 activity; (5) a substantially pure antibody preparation comprising an antibody raised against a SID-1 polypeptide; (6) kits for detecting: (a) at least a **portion** of a SID-1 nucleic acid comprising (I), and a means for detecting the isolated nucleic acid; or (b) at least an epitope of a SID-1 protein comprising the anti-SID-1 antibody of (2), and a means for detecting the antibody; and (7) methods for **reducing** the **expression** of a **target** gene in a cell, a population of cells, or an animal.

BIOTECHNOLOGY - Preferred Protein: The polypeptide comprises: (a) the amino acid sequence of P1; or (b) a sequence comprising a polypeptide encoding residues 19-341, 314-339, 425-451, 481-502, 509-541, 546-571, 575-599, 601-621, 633-655, 659-681, 692-712, or 742-766 of the sequence of P1. Preferred Kit: The means for detecting the isolated nucleic acid comprises a detectable label bound to it, or a labeled secondary nucleic acid that hybridizes to the isolated nucleic acid. The means for detecting the anti-SID-1 antibody comprises a detectable label bound to it, or a labeled secondary antibody that specifically binds to the anti-SID-1 antibody. Preferred Vector: The nucleic acid is operably joined to an exogenous regulatory region, or to heterologous coding sequences to form a fusion vector. Preferred Cell: In the cell transformed with a genetic construct, the nucleic acid is operably joined to heterologous sequences to encode a fusion protein. The cell is a bacterial cell, yeast cell, insect cell, nematode cell, amphibian cell, rodent cell, or human cell. Preferably, the cell consists of mammalian somatic cells, fetal cells, embryonic stem cells, zygotes, gametes, germ line cells, or transgenic animal cells. Preferred Transgenic Animal: The modification is preferably insertion of a nucleic acid encoding a

TITLE: **Inhibition** of undesirable gene expression with **double-stranded RNA**
INVENTOR(S): Kreutzer, Roland; Limmer, Stephan
PATENT ASSIGNEE(S): Germany
SOURCE: PCT Int. Appl., 57 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000044895	A1	20000803	WO 2000-DE244	20000129
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
DE 19956568	A1	20000817	DE 1999-19956568	19991124
CA 2359180	AA	20000803	CA 2000-2359180	20000129
EP 1144623	A1	20011017	EP 2000-910510	20000129
EP 1144623	B1	20020828		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
EP 1214945	A2	20020619	EP 2002-3683	20000129
EP 1214945	A3	20020904		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL				
AT 222953	E	20020915	AT 2000-910510	20000129
JP 2003502012	T2	20030121	JP 2000-596137	20000129
ES 2182791	T3	20030316	ES 2000-910510	20000129
DE 20023125	U1	20030618	DE 2000-20023125	20000129
ZA 2001005909	A	20020724	ZA 2001-5909	20010718
US 2004053875	A1	20040318	US 2003-382768	20030306
US 2004072779	A1	20040415	US 2003-382395	20030306
US 2004102408	A1	20040527	US 2003-383099	20030306

PRIORITY APPLN. INFO.:
DE 1999-19903713 A 19990130
DE 1999-19956568 A 19991124
EP 2000-910510 A3 20000129
WO 2000-DE244 W 20000129
US 2001-889802 A3 20010917

AB The invention relates to a medicament containing at least one double-stranded oligoribonucleotide (dsRNA) designed to **inhibit** the **expression** of a **target** gene. According to the invention, one strand of the dsRNA is at least in part **complementary** to the target gene. Thus, **inhibition** of model gene expression both in vitro and in vivo (3T3 cells) by **double-stranded RNA** was demonstrated. A 21-nucleotide oligoribonucleotide, to which the **complementary** oligoribonucleotide was covalently attached via a linker, was shown to be an effective **inhibitor**.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 37 OF 37 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:718761 CAPLUS
DOCUMENT NUMBER: 134:203186
TITLE: Gene expression: Total silencing by intron-spliced hairpin RNAs

AUTHOR(S): Smith, Neil A.; Singh, Surinder P.; Wang, Ming-Bo; Stoutjesdijk, Peter A.; Green, Allan G.; Waterhouse, Peter M.
CORPORATE SOURCE: CSIRO Plant Industry, Canberra, ACT 2601, Australia
SOURCE: Nature (London) (2000), 407(6802), 319-320
CODEN: NATUAS; ISSN: 0028-0836
PUBLISHER: Nature Publishing Group
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Post-transcriptional gene silencing (PTGS), a sequence-specific RNA degradation mechanism inherent in many life forms, can be induced in plants by transforming them with either antisense or co-suppression constructs, but typically this results in only a small proportion of silenced individuals. Here we show that gene constructs encoding intron-spliced RNA with a hairpin structure can induce PTGS with almost 100% efficiency when directed against viruses or endogenous genes. Using principles we developed for silencing constructs that express **double-stranded RNA** and inverted-repeat RNA, we made a construct encoding a single self **complementary** hairpin RNA (hpRNA) of the Niaprotease (Pro) gene sequence of potato virus Y (PVY). The construct contains sense and antisense Pro sequences flanking a nucleotide spacer fragment derived from uidaA (GUS) gene. About 60% of the plants that are transformed with the constructs were immune to the virus. In the next experiment, we replaced the spacer with an intron sequence, which is spliced out during pre-mRNA processing to produce loopless hpRNA. As a control, the intron sequence was inserted in the reverse, non-splicing, orientation. When transformed into tobacco, 22 of 34 (65%) reverse-intron plants were immune, a similar frequency to plants transformed with the GUS spacer construct. Amazingly, 22 of 23 plants transformed with the construct containing the functional intron were immune to the virus. This same enhancement was observed when hpRNA constructs against the endogenous .apprch.12-desaturase (Fad2) gene of Arabidopsis, in which 100% (30/30) of plants transformed with the intron construct showed silencing of the gene. The process of intron excision from the construct by spliceosome might help to align the **complementary** arms to the hairpin in an environment favoring RNA hybridization, promoting the formation of a duplex. Alternatively, splicing may transiently increase the amount of hairpin RNA by facilitating, or retarding, the hairpin's passage from the nucleus, or by creating a smaller, **less** nuclease-sensitive loop.

REFERENCE COUNT: 12 THERE ARE 12 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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7 1-5

L7 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2004:355069 CAPLUS
DOCUMENT NUMBER: 140:369914
TITLE: Expression vectors for in situ biosynthesis of dsRNA for use in RNA interference in the treatment of disease
INVENTOR(S): Pachuk, Chaterine J.; Satishchandran, C.; McCallus, Daniel Edward
PATENT ASSIGNEE(S): Nucleonics, Inc., USA
SOURCE: PCT Int. Appl., 204 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004035765	A2	20040429	WO 2003-US33466	20031020

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2002-419532P P 20021018
US 2002-421757P P 20021028

AB Expression vectors for use in the in situ formation of double-stranded RNA (dsRNA) structures for use in the regulation of expression of a target gene are described. Forming the dsRNA in a target cell may avoid the adverse effects of RNA stress associated with systemic administration of the dsRNA. These methods can be used to prevent or treat a disease or infection by silencing a gene associated with the disease or infection. The invention also provides methods for identifying nucleic acid sequences that modulate a detectable phenotype, such as the function of a cell, the expression of a gene, or the biol. activity of a target polypeptide.

L7 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2004:80339 CAPLUS
DOCUMENT NUMBER: 140:140621
TITLE: Gene-targeted double stranded RNA for attenuating gene expression
INVENTOR(S): Beach, David; Bernstein, Emily; Caudy, Amy; Hammond, Scott; Hannon, Gregory
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 55 pp., Cont.-in-part of Appl. No. PCT/US01/08435.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 5
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2004018999	A1	20040129	US 2001-858862	20010516
WO 2001068836	A2	20010920	WO 2001-US8435	20010316

WO 2001068836 A3 20020314
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
US 2004086884 A1 20040506 US 2003-350798 20030124
PRIORITY APPLN. INFO.: US 2000-189739P P 20000316
US 2000-243097P P 20001024
WO 2001-US8435 A2 20010316
US 2001-858862 A2 20010516
US 2001-866557 A2 20010524
US 2002-55797 A2 20020122

AB The present invention provides methods for attenuating gene expression in a cell using gene-targeted double stranded RNA (dsRNA). The dsRNA contains a nucleotide sequence that hybridizes under physiol. conditions of the cell to the nucleotide sequence of at least a portion of the gene to be inhibited (the "target" gene).

L7 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:334707 CAPLUS
DOCUMENT NUMBER: 138:358408
TITLE: Gene targeting methods using dsRNA for RNA interference with gene expression and their therapeutic use
INVENTOR(S): Beach, David; Bernstein, Emily; Caudy, Amy; Hammond, Scott; Hannon, Gregory
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 74 pp., Cont.-in-part of Appl. No. PCT/US01/08435.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 5
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003084471	A1	20030501	US 2002-55797	20020122
WO 2001068836	A2	20010920	WO 2001-US8435	20010316
WO 2001068836	A3	20020314		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
WO 2003062394	A2	20030731	WO 2003-US1963	20030122
WO 2003062394	C2	20031002		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG,				

CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

US 2004086884 A1 20040506 US 2003-350798 20030124

PRIORITY APPLN. INFO.: US 2000-189739P P 20000316
US 2000-243097P P 20001024
WO 2001-US8435 A2 20010316
US 2001-858862 A2 20010516
US 2001-866557 A2 20010524
US 2002-55797 A 20020122

AB The present invention provides methods for attenuating gene expression in a cell using gene-targeted double stranded RNA (dsRNA). The dsRNA contains a nucleotide sequence that hybridizes under physiol. conditions of the cell to the nucleotide sequence of at least a portion of the target gene to be inhibited. The dsRNA may be used in gene therapy.

L7 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:833576 CAPLUS
DOCUMENT NUMBER: 137:358079
TITLE: Methods and compositions for RNA interference
INVENTOR(S): Beach, David; Bernstein, Emily; Caudy, Amy; Hammond, Scott; Hannon, Gregory
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 56 pp., Cont.-in-part of Appl. No. PCT/US01/08435.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 5
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002162126	A1	20021031	US 2001-866557	20010524
WO 2001068836	A2	20010920	WO 2001-US8435	20010316
WO 2001068836	A3	20020314		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

US 2004086884 A1 20040506 US 2003-350798 20030124

PRIORITY APPLN. INFO.: US 2000-189739P P 20000316
US 2000-243097P P 20001024
WO 2001-US8435 A2 20010316
US 2001-858862 A2 20010516
US 2001-866557 A2 20010524
US 2002-55797 A2 20020122

AB The present invention provides methods for attenuating gene expression in a cell using gene-targeted double stranded RNA (dsRNA). The dsRNA contains a nucleotide sequence that hybridizes under physiol. conditions of the cell to the nucleotide sequence of at least a portion of the gene to be inhibited (the "target" gene).

L7 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:693495 CAPLUS
DOCUMENT NUMBER: 135:252745
TITLE: Methods for attenuation of gene expression in a cell using RNA interference method
INVENTOR(S): Beach, David; Bernstein, Emily; Caudy, Amy; Hammond,

Scott; Hannon, Gregory
PATENT ASSIGNEE(S): Genetica, Inc., USA; Cold Spring Harbor Laboratory
SOURCE: PCT Int. Appl., 135 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 5
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001068836	A2	20010920	WO 2001-US8435	20010316
WO 2001068836	A3	20020314		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1272630	A2	20030108	EP 2001-918752	20010316
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
JP 2003526367	T2	20030909	JP 2001-567320	20010316
US 2004018999	A1	20040129	US 2001-858862	20010516
US 2002162126	A1	20021031	US 2001-866557	20010524
US 2003084471	A1	20030501	US 2002-55797	20020122
US 2004086884	A1	20040506	US 2003-350798	20030124

PRIORITY APPLN. INFO.:

US 2000-189739P	P	20000316
US 2000-243097P	P	20001024
WO 2001-US8435	W	20010316
US 2001-858862	A2	20010516
US 2001-866557	A2	20010524
US 2002-55797	A2	20020122

AB The present invention provides methods for attenuating gene expression in a cell using gene-targeted double stranded RNA (dsRNA). The dsRNA contains a nucleotide sequence that hybridizes under physiol. conditions of the cell to the nucleotide sequence of at least a portion of the gene to be inhibited (the "target" gene). The invention also provides protein and cDNA sequences of **Dicer** and or **Argonaut** polypeptides of human and Drosophila melanogaster. In preferred embodiments, the present invention provides for ectopic activation of **Dicer**.

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cellular function for the RNA-interference enzyme **Dicer** in the maturation of the let-7 small temporal RNA

AUTHOR(S): Hutvagner, Gyorgy; McLachlan, Juanita; Pasquinelli, Amy E.; Balint, Eva; Tuschl, Thomas; Zamore, Phillip D.

CORPORATE SOURCE: Department of Cellular Biochemistry, Max-Planck-Institute for Biophysical Chemistry, Gottingen, D-37077, Germany

SOURCE: Science (Washington, DC, United States) (2001), 293(5531), 834-838
CODEN: SCIEAS; ISSN: 0036-8075

PUBLISHER: American Association for the Advancement of Science

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The 21-nucleotide small temporal RNA (stRNA) let-7 regulates developmental timing in Caenorhabditis elegans and probably in other bilateral animals. We present in vivo and in vitro evidence that in Drosophila melanogaster a developmentally regulated precursor RNA is cleaved by an RNA interference-like mechanism to produce mature let-7 stRNA. Targeted destruction in cultured human cells of the mRNA encoding the enzyme **Dicer**, which acts in the RNA interference pathway, leads to accumulation of the let-7 precursor. Thus, the RNA interference and stRNA pathways intersect. Both pathways require the RNA-processing enzyme **Dicer** to produce the active small-RNA component that represses gene expression.

REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L1 ANSWER 605 OF 614 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2001:586356 CAPLUS

DOCUMENT NUMBER: 136:212341

TITLE: Dicing up RNAs

AUTHOR(S): Ambros, Victor

CORPORATE SOURCE: Dep. Genetics, Dartmouth Med. Sch., Hanover, NH, 03755, USA

SOURCE: Science (Washington, DC, United States) (2001), 293(5531), 811, 813
CODEN: SCIEAS; ISSN: 0036-8075

PUBLISHER: American Association for the Advancement of Science

DOCUMENT TYPE: Journal; General Review

LANGUAGE: English

AB A review with refs. presents evidence that gene inactivation by RNA interference (RNAi), and the control of developmental timing, are interconnected processes that share certain mol. components. The most prominent of these shared components is the highly conserved nuclease **Dicer**, which cleaves double-stranded RNA precursor mols. into small temporal RNA (stRNA) and short interfering RNA (siRNA). Studies by Zamore et al. (2000) indicate that the RNase III-like enzyme **Dicer** is the central connection between RNAi and stRNAs. **Dicer** particularly recognizes the ends of dsRNA mols. and bites off ≈22 nucleotide chunks as it moves along the mol. These ≈22-nucleotide **Dicer** products become siRNAs when processed from a long dsRNA precursor, or stRNAs when processed from a let-7 or lin-4 RNA precursor. Proof that **Dicer activity** is required for RNAi comes from studies where loss of **Dicer** by depleting Drosophila cells of the protein or inactivating the dcr-1 gene in worms results in the disabling of RNAi. These new finding support the notion that siRNAs and stRNAs are different facets of one diversified system for RNA-mediated gene regulation.

REFERENCE COUNT: 16 THERE ARE 16 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L1 ANSWER 606 OF 614 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2001:578373 CAPLUS
DOCUMENT NUMBER: 135:132810
TITLE: RNA interference (RNAi): gene suppression by dsRNA
AUTHOR(S): Ushida, Chisato
CORPORATE SOURCE: Fac. Agric. Life Sci., Hirosaki Univ., Japan
SOURCE: Tanpakushitsu Kakusan Koso (2001), 46(10), 1381-1386
CODEN: TAKKAJ; ISSN: 0039-9450
PUBLISHER: Kyoritsu Shuppan
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese
AB A review with 30 refs., on mol. mechanism of RNA interference (RNAi), post-transcriptional gene suppression by dsRNA, discussing identification of RNAi-related genes, discovery of short interference RNA (siRNA) involved in RNAi, 2 RNase activities, i.e., dsRNA cleavage by **Dicer** and cleavage of target mRNA by RISC (RNA-induced silencing complex), and action mechanism of RNAi.

L1 ANSWER 607 OF 614 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:544942 CAPLUS
DOCUMENT NUMBER: 135:193142
TITLE: Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control C. elegans developmental timing
AUTHOR(S): Grishok, Alla; Pasquinelli, Amy E.; Conte, Darryl; Li, Na; Parrish, Susan; Ha, Ilho; Baillie, David L.; Fire, Andrew; Ruvkun, Gary; Mello, Craig C.
CORPORATE SOURCE: Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA, 01605, USA
SOURCE: Cell (Cambridge, MA, United States) (2001), 106(1), 23-34
CODEN: CELLB5; ISSN: 0092-8674
PUBLISHER: Cell Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB RNAi is a gene-silencing phenomenon triggered by double-stranded (ds) RNA and involves the generation of 21 to 26 nt RNA segments that guide mRNA destruction. In Caenorhabditis elegans, lin-4 and let-7 encode small temporal RNAs (stRNAs) of 22 nt that regulate stage-specific development. Here inactivation of genes related to RNAi pathway genes, a homolog of Drosophila **Dicer** (dcr-1), and two homologs of rde-1 (alg-1 and alg-2), cause heterochronic phenotypes similar to lin-4 and let-7 mutations. Further dcr-1, alg-1, and alg-2 are necessary for the maturation and activity of the lin-4 and let-7 stRNAs. The authors' findings suggest that a common processing machinery generates guide RNAs that mediate both RNAi and endogenous gene regulation.
REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L1 ANSWER 608 OF 614 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:66972 CAPLUS
DOCUMENT NUMBER: 134:248686
TITLE: Role for a bidentate ribonuclease in the initiation step of RNA interference
AUTHOR(S): Bernstein, Emily; Caudy, Amy A.; Hammond, Scott M.; Hannon, Gregory J.